

Breeding Pearl Millet Male-Sterile Lines

K.N. Rai¹ and N.B. Singh²

Abstract

Numerous sources of cytoplasmic-genic male-sterility are now available in pearl millet. Although an understanding of the genetic control of male-sterility in pearl millet is far from clear, research indicates that it is due to an interaction between sterility-inducing factor or factors in the cytoplasm and multiple major genes and modifiers in the nucleus. Environmental factors, such as temperature and relative humidity, are also assumed to affect the expression of male-sterility. Tift 23A₁ cytoplasm has been extensively used to breed diverse male-sterile lines at several leading research centers because of its more stable sterility across seasons and sites, and because there are a number of agronomically good lines that can be used as donors. Since the Tift 23A₁ cytoplasm has been shown not to be associated with susceptibility to downy mildew, genetic diversification with this cytoplasm will continue to be a major objective in breeding male-sterile lines.

In most male-sterile breeding programs the common objectives will be to breed for dwarf plant height, early to medium maturity, large seeds, improved seed yield and combining ability, stable sterility, and appropriate combinations of resistance to diseases (downy mildew, smut, and ergot). Characterization of the nature and magnitude of cytoplasmic diversity should receive increased attention to identify alternative sources and systems to diversify the cytoplasmic base of male-sterile lines. Better understanding of the inheritance of male-sterility and the environmental factors affecting its expression will contribute significantly to the efficient utilization of diverse genetic materials in breeding male-sterile lines, as well as to the management of male-sterile lines under commercial production.

Résumé

Sélection de lignées mâles-stériles du mil : Il existe plusieurs sources de stérilité mâle cytoplasmique-génique chez le mil. A l'état actuel de nos connaissances encore incomplètes, la stérilité mâle serait due à l'interaction entre un élément ou éléments provoquant la stérilité présent(s) dans le cytoplasme, d'une part, et de multiples gènes et modificateurs majeurs dans le noyau, d'autre part. Les facteurs environnementaux tels que la température et l'humidité relative auraient également un effet sur l'expression de la stérilité mâle. Plusieurs centres de recherche se servent du cytoplasme Tift 23A₁ pour la sélection de différentes lignées mâles-stériles à cause de la stabilité de ce caractère dans l'espace et le temps et puisqu'il existe plusieurs lignées à bons caractères agronomiques pouvant servir de donneurs. Ce cytoplasme n'étant pas sensible au mildiou, la diversification génétique à partir de ce cytoplasme restera un objectif important dans la sélection de lignées mâles-stériles.

En général, la sélection visera à réduire la hauteur de la plante et la durée de la maturation à un cycle allant de précoce à moyen, à augmenter la taille des grains, tout en améliorant le rendement en grain et l'aptitude à la combinaison. D'autres objectifs sont une stérilité stable et une résistance multiple aux maladies (mildiou, charbon, ergot). L'intensification des études sur la caractérisation de la nature du

1. Millet Breeder, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh 502 324 India.
2. Millet Breeder, Punjab Agricultural University, (PAU), Ludhiana, India.

Submitted as CP 378 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

ICRISAT (International Crops Research Institute for the Semi-Arid Tropics). 1987. Proceedings of the International Pearl Millet Workshop, 7-11 April 1986, ICRISAT Center, India. Patancheru, A.P. 502 324, India: ICRISAT.

cytoplasme ainsi que la détermination de l'amplitude de sa diversité permettront d'identifier d'autres sources et systèmes en vue de diversifier la base cytoplasmique des lignées mâles-stériles. Une meilleure compréhension de l'hérédité de la stérilité mâle et des facteurs environnementaux qui influencent son expression contribuera sensiblement à une exploitation efficace de la diversité génétique dans la sélection des lignées mâles-stériles ainsi que la gestion de ces lignées en production commerciale.

Introduction

The discovery of cytoplasmic-genic male-sterility often called cytoplasmic male-sterility and the development of male-sterile line Tift 23A₁ (Burton 1958, 1965) laid the foundation of the pearl millet (*Pennisetum americanum*) hybrid seed industry in India. The first commercial hybrid was HB 1. It was bred on Tift 23A₁ at the Punjab Agricultural University (PAU), Ludhiana, India, and it showed a 100% yield advantage over the open-pollinated check varieties (Athwal 1966). The success of male-sterile line Tift 23A₁ in producing high-yielding hybrids and their failure to last long due to downy mildew (*Sclerospora graminicola*) epidemics led to intensified research on genetic and cytoplasmic diversification of male-sterile lines, with particular emphasis on downy mildew resistance.

As a result, diverse, downy mildew resistant male-sterile lines, based on Tift 23A₁ cytoplasm, were bred. In the meantime, apparently two additional systems of cytoplasmic male-sterility were also discovered (Burton and Athwal 1967) and utilized for breeding male-sterile lines, mainly at PAU. Recent downy mildew epidemics on the most commonly used male-sterile line, 5141A, and its hybrids which are under extensive commercial utilization has shown, once again, that large scale homogeneity of the genetic base is counter to the longevity of male-sterile lines and their hybrids. It is also clear that downy mildew is a major and unpredictably devastating disease of pearl millet hybrids. Recent research at ICRISAT has shown that ergot (*Claviceps fusiformis*) and smut (*Tolyposporium penicillariae*), although of secondary importance, can be more serious on hybrids than on open-pollinated varieties (Thakur et al. 1983a, 1983c), thus highlighting the need to breed male-sterile lines which also have resistance to ergot and smut.

Details of male-sterile breeding at various centers are not described in this paper as they have been extensively reviewed by Kumar and Andrews (1984). Instead, the approaches followed at the leading centers working on male-sterile lines are summarized, and the usefulness of various male-sterile lines in

terms of their distinctness, use in hybrid production, and downy mildew resistance is evaluated. Although the treatment of the subject matter is based mostly on male-sterile breeding work done at Tifton, Georgia, USA, and in India, it is hoped that the principles and the materials described in this paper will find applications elsewhere.

Genetic Diversification and Utilization of A₁ System Male-Sterile L⁻

The A₁ cytoplasm of Tift 23A₁ has been extensively utilized in breeding a wide range of male-sterile lines (Table 1). At Tifton, four male-sterile lines were produced either by transferring single genes for specific traits into Tift 23A₁ and Tift 23B, or by backcrossing nonrestorers into Tift 23A₁ cytoplasm. Of these, mostly Tift 23A₁, and to some extent Tift 23DA₁, were used in India for hybrid production. Hybrids on Tift 23A₁ yielded more than those on Tift 23DA₁; five hybrids on Tift 23A₁ were released (Table 1) in quick succession between 1965-1972, but all went out of cultivation within about 5 years of their release due to high downy mildew susceptibility.

When hybrids on Tift 23A₁ succumbed to downy mildew, research efforts were intensified mainly at PAU and at the Indian Agricultural Research Institute (IARI), New Delhi, to diversify the genetic base of male-sterile lines in the A₁ cytoplasmic system with high levels of downy mildew resistance. Seventeen male-sterile lines (Pb 111A, Pb 101A, and Pb 2 Pb 215A) which possessed high levels of downy mildew resistance were bred at PAU. They show considerable variability for tillering, head length, and compactness, and are of medium or mid-late maturity. Five of these have been involved in extensive hybrid testing. However, only one (Pb 111A) has successfully produced high-yielding hybrids, of which four have so far been released by the All India Coordinated Millets Improvement Project (AIC-MIP). Two of these (PHB 10 and PHB 14) were the first downy mildew resistant hybrids released in India (Gill et al. 1975).

Table 1. Male-sterile lines of pearl millet released in India based on Tift 23A₁ cytoplasm and grain hybrids.

Research organization	Male-sterile lines	Hybrids ¹	Remarks
Coastal Plain Experiment Station, Tifton, Georgia, USA	Tift 23A ₁ , Tift 23DA ₁ , Tift 23DA ₁ E, Tift 18A	HB 1, HB 2, HB 3, HB 4, HB 5	All hybrids on Tift 23A ₁
Punjab Agricultural University, Ludhiana, India	Pb 111A, Pb 101A, Pb 201-Pb215A	PHB 10, PHB 14, PHB 47, X5	All hybrids on Pb 111A
Indian Agricultural Research Institute, New Delhi, India	5141A, 5054A, 3893A, 3383A, 5071A, 5540A, 5184A	BJ 104, BK 560, BD 111, BD 763, CoH2, HHB 45, GHB 27, GHB 32, CM 46, CJ 104, NHB 3	First 8 hybrids on 5141A; next 2 on 5054A and the last one on 5071A
ICRISAT Patancheru, India	81A, 833A, 834A, 841A, 842A, 843A, 851A, 852A	ICMH 451, ICMH 501	ICMH 451 on 81A, ICMH 501 on 834A
Maharashtra Hybrid Seeds Company, Ltd. Jalna, India	MS1, MS2, MS4-MS9, MS11-MS14	MBH 110, MBH 118, MBH 130, MBH 131, MBH 136	All hybrids on MS2

1. Hybrids and male-sterile lines not necessarily produced by the same research organization.

The work at IARI produced at least seven male-sterile lines (Table 1). However, only three (5054A, 5071A, and 5141A) have good hybrid potential and downy mildew resistance (Pokhriyal et al. 1976). Male-sterile line 5071A was used to reconstitute HB 3 as NHB 3 but it did not become popular because of its susceptibility to downy mildew. The most useful among these male-sterile lines was 5141A on which eight hybrids were released by AICMIP during 1972-1984 (Table 1). Two hybrids (BJ 104 and BK 560) were widely cultivated in India and dominated the hybrid seed industry for about a decade. Two hybrids were released on 5054A but only one, CJ 104, became popular in the drier parts of Gujarat state due to its earliness (75 d to maturity). Male-sterile line 3383A is currently the highest-tillering male-sterile line and it also has a high level of downy mildew resistance. It has very thin heads and small seeds; empirical evidence shows that it is perhaps not a good general combiner.

Maharashtra Hybrid Seeds Company Ltd. (MAHYCO) at Jalna, India, initiated its male-sterile breeding work with S10A and S10B, introduced from Serere, Uganda. MS₂ was the first successful male-sterile line bred from this stock on which two hybrids have been released by AICMIP; two others are promising and are likely to be released. Eleven additional male-sterile lines with different plant

height, head size, and head compactness, but with larger seeds and improved seed set, have been bred by MAHYCO so far. Four of these are promising, and higher-yielding hybrids that have these male-sterile lines as seed parents are in advanced yield tests. MAHYCO has emphasized breeding for large seed size and early to medium maturity.

Starting in the late 1970s, seven male-sterile lines were bred at ICRISAT which have diverse plant height, maturity, tillering, head volume, and seed size. Two of these (81A and 834A) are currently under extensive utilization in the hybrid programs in India. Two hybrids (ICMH 451 on 81A and ICMH 501 on 834A) yielded as high as the best check hybrid MBH 110 in AICMIP trials over years, and were released in 1986 for cultivation throughout India. The parental lines of these hybrids are currently being multiplied by various seed agencies. Because of initial concentration on 81A and 834A, other male-sterile lines have not been extensively tried in hybrid combination. There are indications that 833A and 852A have as good a general combining ability as 81A and 834A. Male-sterile line 841A, selected from the residual variability for downy mildew resistance in 5141A, compares well with the general combining ability of the latter and possesses much improved downy mildew resistance (Singh et al. 1987). Male-sterile lines 842A and 843A, bred

jointly by the Fort Hays Branch Experiment Station, Kansas, USA, and by ICRISAT have large seeds (up to 12 g 1000⁻¹) and mature early. The earliest male-sterile is 843A (42 d to 50% bloom) among all those currently available male-sterile lines in India, and it produces very early, short hybrids. However, it seems that due to downy mildew susceptibility, its use in hybrid production will be very restricted and it will be of negligible commercial value in downy mildew endemic areas.

At present, the major emphasis in India is to breed medium-maturity male-sterile lines (time to bloom, 45-55 d), primarily for two reasons:

- most breeding materials (including composites and synthetics used to produce restorer lines) belong to the medium maturity class and offer a wide range of genetic diversity, and
- the medium maturity class perhaps represents currently the most productive group of materials.

With the extensive use of germplasm from Togo and Ghana and breeding materials from the Fort Hays Branch Experiment Station, a wide range of male-sterile lines in the early maturity group are expected in the near future. For plant height, the emphasis is shifting towards dwarf male-sterile lines. Dwarf lines are considered important because they are, in general:

- less prone to lodging even under the intensive management conditions of seed production,
- are easy to stabilize for height,
- easy to recover in crosses,
- provide an option to produce hybrids with a wide height range, and
- make more efficient use of pollen from restorers in hybrid seed production plots.

Cytoplasmic Diversification of Male-Sterile Lines

Enormous differences in downy mildew incidence among male-sterile lines based on Tift 23A₁ cytoplasm indicate that the cytoplasm is not associated with downy mildew susceptibility, and that it is nuclear gene resistance which is important. Experimental evidence confirms this assumption (Kumar et al. 1983). Thus, at present, there is no need to be alarmed about the vulnerability of Tift 23A₁ cytoplasm to downy mildew. However, in the long run, the large scale and continuous use of a single cyto-

plasm source runs the risk of it becoming vulnerable to existing or unforeseen diseases. Hence, there is a need to diversify the cytoplasmic base of male-sterile lines. The cytoplasmic diversification should encompass other sources of cytoplasm within the A₁ system, as well as other cytoplasmic systems.

In 1961, a cytoplasmic male-sterile line was identified at PAU (Athwal 1961) in a very late-maturing genetic stock, IP 189. The sterile plants were pollinated with different sources of maintainers and the male-sterile line finally produced was called CMS 66A (L 66A). In 1962, male-sterile plants were observed at PAU in a population originating from a natural cross of a stock possessing pearly amber grains. The cytoplasmic male-sterile line bred using this source was called CMS 67A (L 67A) (Athwal 1966). Burton and Athwal (1967) compared the relationships between the cytoplasmic systems of Tift 23A₁, L 66A and L 67A in experiments conducted at Tifton and Ludhiana and concluded that these three represented three different systems of cytoplasmic male-sterility. Several male-sterile lines were produced with these new cytoplasmic systems. Burton and Athwal (1969) produced Tift 239DA₂ by backcrossing Tift 239DB into L 103A (an A₂-system male-sterile line). At PAU, nine male-sterile lines were bred: (Pb 301A-Pb 309A) with A₂ cytoplasm and four male-sterile lines (Pb 401A-Pb 403A and Pb 405A) with A₃ cytoplasm. Most of these male-sterile lines are resistant to downy mildew and have quite diverse phenotypic characters. A high-yielding hybrid (PHB 108) has recently been identified on Pb 405A₃. It yielded as much as the best check hybrid MBH 110 in the AICMIP hybrid trials in 1984 and 1985.

Several other sources of cytoplasmic male-sterility have recently been reported. Cytoplasmic male-sterility has been identified in four pearl millet accessions from the ICRISAT genetic resources collection (S. Appa Rao, ICRISAT, personal communication) and there are indications that they resemble the A₃ system. None of these, however, has been stable for sterility: under selfing, partial fertility was observed in some plants which have 1-10 grains per head. Two other sources of cytoplasmic male-sterility have been reported (Appadurai et al. 1982, Aken'Ova and Chheda 1981), but it has not been conclusively shown whether they are different from the other existing systems. Marchais and Pernès (1985) have recently reported an interesting source of cytoplasmic male-sterility discovered from a cross between a wild relative of pearl millet [*Pennisetum violaceum* (Lam.) L. Rich = *P. americanum* ssp. *monodii*] and a landrace cultivar (Tiotande) from

Senegal. This source is reported to be different from all the existing systems. In a general survey of the germplasm and breeding lines using this male-sterile line, Marchais and Pernès (1985) showed that the frequency of restorer alleles was generally low in the cultivated millets, whatever their origin, and high in wild millets. This points clearly to the immediate utility of this cytoplasm in male-sterile breeding and the utility of wild species which have a high frequency of restorers for breeding pollinations.

Studies of diallel F_1 hybrids among several A and B lines, all incorporating Tift 23A₁ cytoplasm, did not clearly show that a maintainer of a given male-sterile line was necessarily a maintainer on the other male-sterile lines (Table 2). This shows that there is perhaps a continuum between different cytoplasmic systems which involves complex genetic mechanisms of multigenic inheritance confounded with modifying genes and environmental factors. Field evaluation of cytoplasmic diversity based on the fertility response of F_1 hybrids between cytoplasm sources and a set of inbreds may, therefore, suffer from these confounding effects as shown in sorghum (Ross and Hackerott 1972, Conde et al. 1982). Thus biochemical techniques such as those used in sorghum, in conjunction with field tests (Conde et al. 1982), may be quite valuable to characterize the nature and magnitude of cytoplasmic diversity in pearl millet.

Genetics of Cytoplasmic Male-Sterility

Burton and Athwal (1967) studied the genetics of cytoplasmic male-sterility by crossing Tift 23A₁, L

66A, and L 67A with each of their maintainers and restorers of Tift 23A₁. Based on the fertility/sterility of F_1 hybrids in nurseries grown at Tifton and Ludhiana, it was proposed that:

cytoplasmic male-sterility results from the interaction of a specific recessive gene, *ms*, in the homozygous condition with sterility-inducing factors in the cytoplasm, and different single genes in recessive form are responsible for male-sterility in different cytoplasmic systems.

The genetic model proposed for male-sterile lines, maintainers, and restorers used in their studies is summarized in Table 3. Burton and Athwal (1967) recognized the possible role of modifiers and environmental factors in the maintenance of sterility and restoration of fertility. Siebert (1982) studied the genetics of fertility restoration and suggested there were two dominant complementary genes and a modifier for the A₁ system, and two dominant duplicate factors for the A₂ system. It has also been suggested that the maintenance of sterility of a new cytoplasmic system discovered by Marchais and Pernès (1985) from *P. violaceum* cytoplasm is controlled by three independently inherited recessive genes.

Several progenies derived from B × R crosses in pearl millet when tested on two male-sterile lines (843A and 81A) did not conform to monogenic or digenic inheritance (Table 4). This means that there are more than two major genes involved in the maintenance of sterility, or that there are modifier genes involved. Considerable variability in the expression of sterility in A₁ (Tift 23A₁) × B crosses has been

Table 2. Fertility/sterility of (A×B) F_1 hybrids¹.

Female parent	Male parent						
	842B	843B	81B	5141B	834B	111B	PT732B
842A	S ²	F ³	S	F	F	F	F
843A	F	S	S	- ⁴	F	F	F
81A	S	S	S	S	F	S	F
5141A	F	F	S	S	F	F	F
834A	F	F	F	F	S	F	F
111A	F	F	S	F	F	S	F
PT 732A	F	F	F	F	F	F	S

1. ICRISAT Center data: 1985 rainy season.

2. S = Sterile hybrid.

3. F = Fertile hybrids.

4. - = Not involved in this study.

Table 3. Fertility-sterility in (A×B) and (A×R) F_1 hybrids across three cytoplasmic systems and the genetic constitution of B and R lines.¹

Male parent	Female parent			
	23A ₁	L66A ₂	L67A ₃	Genetic constitution
23B ₁	S	F	F	<i>ms</i> ₁ <i>ms</i> ₁ MS ₂ MS ₂ MS ₃ MS ₃
L66B ₂	S	S	F	<i>ms</i> ₁ <i>ms</i> ₁ <i>ms</i> ₂ <i>ms</i> ₂ MS ₃ MS ₃
L67B ₃	S	S	S	<i>ms</i> ₁ <i>ms</i> ₁ <i>ms</i> ₂ <i>ms</i> ₂ <i>ms</i> ₃ <i>ms</i> ₃
T13R ₁	F	S	S	MS ₂ MS ₂ <i>ms</i> ₂ <i>ms</i> ₂ <i>ms</i> ₃ <i>ms</i> ₃
L4R ₁	F	S	S	MS ₂ MS ₂ <i>ms</i> ₂ <i>ms</i> ₂ <i>ms</i> ₃ <i>ms</i> ₃
L6R	F	F	F	MS ₂ MS ₂ MS ₃ MS ₃ MS ₃ MS ₃

1. Modified from Burton and Athwal (1967). B lines have normal (N) cytoplasm; A lines have sterile (A) cytoplasm.

Table 4. Frequency of maintainer progenies in (B×B) and (B×R) crosses when tested on two male-sterile line testers.¹

Cross	Tester	No. of progenies				B (%)
		Total	R	B	Others ²	
(B×B) Cross ³	81A	85	0	69	16	81
	843A	64	6	12	46	19
(B×R) Crosses ⁴	81A	46	43	0	3	0
	843A	34	29	0	5	0

1. Based on two seasons' data at ICRISAT Center.
2. Includes those progenies which were not consistently restorer (R) or maintainer (B) in both seasons.
3. Progenies from two populations on tester 81A and four populations on tester 843A.
4. Progenies from two populations on each tester.

observed. Forty nine $A_1 \times B$ crosses involving six A lines with Tift 23A₁ cytoplasm, one with PT 732A (probably non- A_1) cytoplasm, and their respective maintainers were studied for pollen shed and seed set (under selfing) at ICRISAT Center (Table 2). The pollen shed and seed set data were in good agreement: F_1 s shedding pollen gave good seed set and the F_1 s producing no pollen failed to set seed under selfing. This study showed that:

- the maintainer of a given A_1 system male-sterile line was not necessarily a maintainer on the other A_1 system male-sterile lines;
- all the A_1 system maintainers except 834B were maintainers on 81A, and 81B was maintainer on all the A_1 system male-sterile lines except 834A; and
- 834B and PT 732B were restorers on other male-sterile lines except on their own respective male-sterile lines (Table 2).

These points imply the possible involvement of multiple major genes and modifiers in determining the male-sterility.

Studies in sorghum show that inheritance patterns of cytoplasmic male-sterility vary widely from one group of materials to the other. For instance, Stephens and Holland (1954) postulated more than two pairs of genes whereas Maunder and Pickett (1959) presented evidence for a single gene control. Based on pollen viability studies, Pi and Wu (1961) found indications of three types of genetic control: single gene control in five crosses, two gene control in two crosses, and complex inheritance in another two crosses. Alam and Sandal (1967) also found evi-

dence for single gene control in three crosses, two gene control in two crosses, and three gene control in one cross in sudangrass (*Sorghum vulgare* var. *sudanense*). In an extensive study in sorghum, Appadurai and Ponnaiya (1967) found cytoplasmic male-sterility under single gene control only in 4 out of 11 crosses; in the remaining 7 crosses, the inheritance appeared to be rather complex.

Breakdown of Male-Sterility

A few heads shed pollen from the nodal tillers of Tift 23A₁ (Rao 1969). Burton (1972) made detailed studies of fertile sectors in Tift 23A₁ heads and observed that the reversion to fertility could be due to nuclear mutation from recessive maintainer allele (ms) to dominant restorer allele (MS), as well as cytoplasmic mutation from sterile (A) to normal (N) cytoplasm. Further studies showed that about 1% of such reversions to fertility are mutations from (A) cytoplasm to (N) cytoplasm, and only about 3% are nuclear mutations from ms to MS allele (Burton 1977). Such reversions were observed both in A_1 and A_2 system male-sterile lines. Clement (1975) studied four male-sterile lines and found all reversions to fertility were mutational changes in the cytoplasm. He also observed up to 30-fold differences in reversion rates between lines (0.03/100 heads in ASM 3 to 1.02/100 heads in ASM 7), indicating the effect of genetic background on the stability of sterility-inducing factors in the cytoplasm. Singh and Laughnan (1972) found in maize that reversions to fertility were all due to changes in cytoplasm from S type to N type, and that there were large differences among genotypes for the rates of such changes.

Thus, it is clear that more than 97% of the reversions to fertility in male-sterile lines are due to changes in the cytoplasm from the sterile to the normal state. These changes would simply produce B-line equivalents, which would cause no problem by pollinating the plants of A-line. However, if reverted fertile plants in A-lines should be rogued to insure pure A-line stock at harvest for subsequent seed increase and hybrid seed production. The reversions to fertility due to nuclear mutations from ms to MS allele, although very rare, can cause considerable problems not only by contaminating A-line plants (whose progenies will also be fertile) but also by contaminating B-line plants, which in turn would no longer serve as maintainers. Limiting the number of generations increased from breeder seeds and timely roguing have been recommended as the best

possible solutions to produce high quality A-line seed for hybrid production (Burton 1977). There is some evidence to show that higher temperatures and low relative humidity lead to a breakdown of male-sterility in pearl millet (Reddy and Reddy 1970, Saxena and Chaudhary 1977). Thus, multiplication of seed of male-sterile lines in areas with lower temperatures (<30°C) and high relative humidity are likely to significantly reduce the problem of pollen shedders in male-sterile lines.

Breeding for Disease Resistance

Downy Mildew Resistance

The longevity of some very promising and widely used male-sterile lines (Tift 23A₁ and 5141A) in the Indian hybrid program was severely reduced by downy mildew susceptibility. Thus, downy mildew resistance continues to be a major objective in male-sterile breeding. Several attempts made to induce resistance or to exploit residual variability for resistance in the susceptible male-sterile lines with high general combining ability have met with varying degrees of success.

At IARI, Tift 23B was irradiated with gamma rays and subsequent selection of progeny produced 5071A, (Murty et al. 1975) which had short-lived resistance to downy mildew. Male-sterile line 81A was bred by irradiating Tift 23DB and selecting the progeny in the disease nursery at ICRISAT. In tests in the disease nursery at ICRISAT Center (Kumar et al. 1984), 81A showed <2% downy mildew as compared to 100% in Tift 23A₁. Under high disease pressure, 81A had 8% downy mildew as compared to 97% in the susceptible check hybrid NHB 3 (Table 5).

The exploitation of residual variability for downy mildew resistance in the otherwise susceptible but promising male-sterile lines and their maintainers has also produced resistant lines with negligible changes in phenotypic characteristics. Selection in Tift 23A₁ and Tift 23B produced resistant male-sterile line Pb 204A at PAU (Gill et al. 1981). Selection in susceptible 5141A produced Pb 211A at Ludhiana and 841A at ICRISAT Center. Pb 211A had 17% downy mildew as compared to 1% in 841A and 28% in unselected original stock of 5141A. (S.D. Singh, ICRISAT, personal communication). Selection in three susceptible male-sterile lines and their maintainers (AKM 2021/BKM 2021, AKM 2026/BKM 2026, AKM 2068/BKM 2068) bred at the Fort

Table 5. Downy mildew incidence in male-sterile lines in downy mildew nursery, ICRISAT Center, rainy season 1984.

Male-sterile line	No. of plants		Downy mildew (%)	Status
	Total	DM infected		
81A	264	20	8	AICMIP release 1986
833A	277	1	>1	AICMIP use since 1983
834A	272	0	0	AICMIP release 1986
841A	245	3	1	AICMIP use since 1984
842A	114	1	1	AICMIP use since 1983
843A	173	20	12	AICMIP use since 1983
852A	380	2	<1	AICMIP test in 1985
111A	249	4	2	AICMIP release, early 70s
5141A	329	153	47	AICMIP release, early 70s
NHB 3	255	249	97	-

K.N. Rai and S.D. Singh, personal communication.

Hays Branch Experiment Station, Kansas, USA, was undertaken at ICRISAT Center. The selection was most effective in AKM 2021/BKM 2021 which produced male-sterile line 842A with 1% downy mildew (Table 5) as compared to about 35% in the AKM 2021/BKM 2021. The selection was least effective in AKM 2026/BKM 2026 (D.J. Andrews, University of Nebraska, personal communication).

At ICRISAT, the hybridization between B-lines with high general combining ability and varying levels of downy mildew resistance has recently produced a large number of maintainer progenies which have shown high levels of resistance in preliminary tests. These progenies at present form a very diverse base to breed male-sterile lines of various maturity and phenotypes. Of the several accessions that showed high levels of stable resistance in multilocal tests over years, two (700651 and P 7) were crossed with 843B to transfer the genes for stable resistance into 843B. The derived, agronomically elite B-lines will be tested multilocally for resistance in disease nurseries and, if the resistance is high and stable, the lines will be utilized further in the male-sterile breeding programs.

Smut Resistance

Recent studies have shown that a high level of smut resistance in one parent is generally adequate to produce smut-resistant hybrids (Table 6). Smut resistance is now available in agronomically elite

Table 6. Smut severity (%) in F_1 hybrids between susceptible (S) \times resistant (SRL) lines.

(S \times R) F_1 hybrids	No. of hybrids	Number of hybrids in smut severity class			
		<1	1-5	6-10	>10
81B \times SRL	46	23	12	1	10
843B \times SRL	43	41	2	0	0
843A \times SRL	46	32	10	1	3

1. All the smut-resistant lines (SRL) were either free or had <1% smut; A and B lines had 32-70% smut severity.

lines and composites with formidable genetic diversity. These will be used to breed resistant pollinators to produce resistant hybrids, even in combination with the existing male-sterile lines, all of which are highly susceptible to smut. However, smut-resistant, male-sterile lines will increase the usefulness of existing pollinators (most of which are susceptible to smut) to produce resistant hybrids. The hybridization between 843B (a large-seeded, early-maturing, d_2 dwarf B-line) and smut-resistant lines has generated a wide range of maintainer progenies which have shown <5% downy mildew and smut in preliminary tests. Following this initial success, several resistant lines have now been crossed with 843B to further diversify the genetic base in the breeding program for smut-resistant, male-sterile lines.

Ergot Resistance

There is evidence that ergot resistance is governed by polygenic recessives (Thakur et al. 1983b). There-

fore, ergot-resistant hybrids cannot be bred unless both hybrid parents possess resistance (Table 7). There is further evidence that the source of resistance in both hybrid parents should be as similar as possible to insure a high level of resistance in hybrids (Rai and Thakur, ICRISAT, personal communication). An ergot-resistant line, ICMPE 134-6-9 (a maintainer on 81A), was converted into a male-sterile line. Like many other ergot-resistant lines, it is tall and matures late. Preliminary tests have not shown this male-sterile line to have high hybrid potential. The ergot-resistant lines are not very diverse, hence crossing this male-sterile line with currently available ergot-resistant pollinators is unlikely to exhibit an improved hybrid yield. This calls for the genetic diversification of the male-sterile lines and pollinators while breeding ergot-resistant hybrid parents.

Ergot-resistant lines (ERL) have been crossed with 843B, a d_2 -dwarf, early-maturing, large-seeded maintainer line with a high general combining ability. The F_2 populations and backcross populations [(843B \times ERL) \times ERL] are at present being screened to select short (d_2), large-seeded, early-maturing, and ergot-resistant plants. Breeding ergot-resistant, male-sterile lines is obviously a slow process, since the polygenically-inherited resistance is available in agronomic backgrounds which are generally neither very diverse nor very promising.

Rust Resistance

Numerous sources of rust resistance have now been reported (ICRISAT 1985). However, an S_2 progeny

Table 7. Ergot severity (%) in testcross hybrids based on ergot-resistant pollen parents and ergot-susceptible and ergot-resistant seed parents; ICRISAT Center Ergot Nursery.

Year	Female parent	No. of F ₁ hybrids	Percentage of hybrids in ergot severity class			
			<10	10-20	21-50	>50
Susceptible seed parents						
1980	111A	189	0	0	0	100
	5054A	216	0	0	0	100
	5141A	237	0	0	0	100
Resistant seed parents						
1982	ER F ₄	49	92	8	0	0
	SC ₂ (R)-5-4 (LES) ²	55	45	29	20	0

1. ER = Ergot resistant.

2. LES = Low Ergot Susceptible.

Source: R.P. Thakur and B.S. Talukdar, personal communication.

selected from IP 2696 has recently been identified as having a single dominant gene for rust resistance (Andrews et al. 1985). Further studies have shown this resistance to be stable across locations in India (ICRISAT 1985). Taking advantage of this simple genetic control, programs are underway at ICRISAT Center to introduce it into potential B-lines for breeding rust-resistant, male-sterile lines.

A male-sterile line, 852A, showed a very high level of rust resistance in a nursery at ICRISAT Center. This line is also highly resistant to downy mildew (Table 5) and a very good general combiner. If found stable across locations, the resistance from 852B will be used extensively in the male-sterile breeding program.

Breeding Methods

Breeding Maintainer Lines

Three essential features of a B-line considered useful for conversion into a male-sterile line are:

- high general combining ability,
- ability to produce a completely sterile F_1 hybrid on a male-sterile line when tested across locations and seasons, and
- ability to produce adequate pollen for the maintenance of an A-line under varying seed production conditions.

The evaluation for these traits is generally undertaken when the B-lines have become highly homozygous. Depending on the agroecological conditions at the location where the hybrids based on a male-sterile line will be cultivated, selection for numerous traits (e.g., downy mildew resistance, maturity, and plant height), are made in preceding generations. Selection for several other characters would seem quite desirable. These include preliminary evaluation of sterile F_1 hybrids made on an A-line to discard those which are not nonrestorers, and selection for high tillering, head volume, head compactness, medium to large seed size, moderate dormancy, resistance to grain weathering, good ear exertion, lodging resistance, and good seed set. This comprehensive list of traits does not exclude consumer quality traits and resistance to other diseases, if these form part of a male-sterile breeding program.

Hard data do not exist to provide guidelines whether to follow recurrent selection or a classic pedigree breeding program. At the moment it seems

that various forms of pedigree breeding used by almost all the major breeding programs have proved quite effective in producing and maintaining a wide range of useful, diverse B-lines. Recurrent selection to breed B-lines initially started with three composites in 1975 at Punjab Agricultural University, Ludhiana. These composites, however, have not been sufficiently exploited to breed B-lines. Because two of these composites have a narrow genetic base, all three have recently been merged, and another 30 B-lines added to widen the genetic base of the resulting composite.

In the long term, it would appear sensible for any breeding program to pursue both methods, but with the emphasis on pedigree breeding.

Combining Ability of Maintainer Lines

During generation advance, mild selection pressure is exerted at each inbreeding stage for the performance per se of the progenies. Although phenotypic performance is important, what matters more within the framework of agronomic acceptability, is the general combining ability of the B-lines. The published records do not show that B-lines in any pearl millet breeding program were tested for combining ability before embarking on their conversion into male-sterile lines. This explains to a large extent why many male-sterile lines bred after 5141A was released have not shown any better hybrid potential than 5141A.

Topcross tests have been recommended as the most practical approach to survey the combining ability of lines when the number of lines under test is too large (Simmonds 1979). No "universal" broad-based tester in pearl millet has yet been found. Limited data available at present show that combining ability of lines may largely depend on the tester (first author's unpublished data), and that perhaps the average of general combining ability estimates based on 3-4 broad-based testers may be much more reliable.

Conversion of Maintainers into Male-Sterile Lines

Maintainers are converted into male-sterile lines by conventional backcross breeding in which a maintainer is used as a recurrent male parent and a male-sterile line as a nonrecurrent parent. At least six backcrosses are required to insure acceptable sim-

ilarity between maintainers and male-sterile lines. Where the maintainers and the donor male-sterile lines (source of sterile cytoplasm) are very different, 1-2 additional backcrosses may be required to achieve acceptable phenotypic similarity between the B- and A-line. Segregation and attendant genetic variation within the progenies in backcross series are expected. This would provide an opportunity for phenotypic selection of individual plants resembling the phenotype of maintainer line. Phenotypic selection and maintaining A/B pairs during the backcrossing will accelerate the conversion process.

Future Directions

Breeding. Breeding early or medium-maturing male-sterile lines with short plants, medium to large seeds, and a good balance between tillering and head volume should form the primary objectives in most breeding programs. Early male-sterile lines would be particularly useful to breed hybrids intended to be grown in areas with likely terminal drought stress or intended to fit in multiple cropping systems. Increasing the yielding ability of male-sterile lines per se, improved general combining ability, and high levels of stable downy mildew resistance (for downy mildew endemic areas) should be an integral part of any male-sterile breeding program. Although of secondary importance, incorporating smut and ergot resistance should also be attempted to mitigate the yield losses from these two head diseases.

Cytoplasmic Diversity. Since evidence suggests there is no relationship between Tift 23A₁ cytoplasm and downy mildew, genetic diversification with Tift 23A₁ cytoplasm can continue. However, to avoid any catastrophic diseases from cytoplasmic uniformity, alternative sources or systems of cytoplasmic male-sterility should be utilized. Various sources of cytoplasm currently available should be characterized for the nature and magnitude of cytoplasmic diversity through the application of biochemical techniques. At the same time, the search should continue for alternative sources of cytoplasm in accessions and in segregating populations derived from divergent crosses. Attempts should also be made to induce cytoplasmic male-sterility as already reported in pearl millet (Burton and Hanna 1982) to diversify the cytoplasmic base of male-sterile lines.

Environmental Factors. The inheritance of cytoplasmic male-sterility and fertility restoration under

varying environmental conditions should be studied. The effects of environmental factors, particularly temperature regimes and humidity levels, on the breakdown of male-sterility should also be examined.

Isonuclear Lines. Isonuclear lines should be created and used to study the effects of different sources and systems of cytoplasmic male-sterility on various plant characters and disease incidence.

References

- Aken'Ova, M.E., and Chheda, H.R. 1981. A new source of cytoplasmic-genic male sterility in pearl millet. *Crop Science* 21:984-985.
- Alam, S., and Sandal, P.C. 1967. Inheritance of cytoplasmic male sterility in sudangrass. *Crop Science* 7:668-671.
- Andrews, D.J., Rai, K.N., and Singh, S.D. 1985. A single dominant gene for rust resistance in pearl millet. *Crop Science* 25:565-566.
- Appadurai, R., and Ponnaiya, B.W.X. 1967. Inheritance of fertility restoration in Sorghum (Moench). *Madras Agricultural Journal* 54:393-404.
- Appadurai, R., Raveendran, T.S., and Nagarajan, C. 1982. A new male-sterility system in pearl millet. *Indian Journal of Agricultural Sciences* 52:832-834.
- Athwal, D.S. 1961. Recent developments in the breeding and improvement of bajra (pearl millet) in the Punjab. *Madras Agricultural Journal* 48:18-19 (Abstract.)
- Athwal, D.S. 1966. Current plant breeding research with special reference to *Pennisetum*. *Indian Journal of Genetics and Plant Breeding* 26A:73-85.
- Burton, G.W. 1958. Cytoplasmic male-sterility in pearl millet (*Pennisetum glaucum* (L.) R. Br. *Agronomy Journal* 50:230.
- Burton, G.W. 1972. Natural sterility maintainer and fertility restorer mutants in Tift 23A₁, cytoplasmic male-sterile pearl millet, *Pennisetum typhoides* (Burm.) Stapf & Hubb. *Crop Science* 12:280-282.
- Burton, G.W. 1965. Pearl millet Tift 23A₁ released. *Crops and Soils* 17:19.
- Burton, G.W. 1972. Natural sterility maintainer and fertility restorer mutants in Tift 23A₂, cytoplasmic male-sterile pearl millet, *Pennisetum typhoides* (Burm.) Stapf & Hubb. *Crop Science* 12:280-282.
- Burton, G.W. 1977. Fertile sterility maintainer mutants in cytoplasmic male sterile pearl millet. *Crop Science* 17:635-637.

- Burton, G.W., and Athwal, D.S. 1967. Two additional sources of cytoplasmic male sterility in pearl millet and their relationship to Tift 23A₁. *Crop Science* 7:209-211.
- Burton, G.W., and Athwal, D.S. 1969. Registration of pearl millet inbreds Tift 239DB₂ and Tift 239DA₂. *Crop Science* 9:398.
- Burton, G.W., and Hanna, W.W. 1982. Stable cytoplasmic male sterile mutants induced in Tift 23DB1 pearl millet with mitomycin and streptomycin. *Crop Science* 22:651-652.
- Clement, W.M., Jr. 1975. Plasmon mutations in cytoplasmic male-sterile pearl millet, *Pennisetum typhoides*. *Genetics* 79:583-588.
- Conde, M.F., Pring, D.R., Schertz, K.F., and Ross, W.M. 1982. Correlation of mitochondrial DNA restriction endonuclease patterns with sterility expression in six male-sterile sorghum cytoplasms. *Crop Science* 22:536-539.
- Gill, K.S., Phul, P.S., and Jindia, L.N. 1975. New bajra hybrids resistant to the downy mildew green ear disease. *Seeds & Forms* 1(7):3-4.
- _____, K.S., Phul, P.S., Jindia, L.N., and Singh N.B. 1981. Pb204A a new downy mildew resistant male-sterile line in pearl millet. *Seeds and Farms* 7:19-20.
- Kumar, K.A., and Andrews, D.J. 1984. Cytoplasmic male sterility in pearl millet (*Pennisetum americanum* (L.) Leeke) a review. *Advances in Applied Biology* 10:113-143.
- Kumar, K.A., Andrews, D.J., Jain, R.P., and Singh, S.D. 1984. ICMA-1 and ICMB-1 pearl millet parental lines with A₁ cytoplasmic-genic male sterility system. *Crop Science* 24:832.
- Kumar, K.A., Jain, R.P., and Singh S.D. 1983. Downy mildew reactions of pearl millet lines with and without cytoplasmic male-sterility. *Plant Disease* 67:663-665.
- ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) 1985. Progress Report: MP-113. Report of the 1984 IPMRN. Patancheru, A.P. 502 324, India: ICRISAT. (Limited distribution.)
- Marchais, L., and Pernès, J. 1985. Genetic divergence between wild and cultivated pearl millets (*Pennisetum phoides*). 1. Male sterility. *Zeitschrift fuer Pflanzen-echtung* 95:103-112.
- Maunder, A.B., and Pickett, R.C. 1959. The genetic inheritance of cytoplasmic genetic male sterility in grain sorghum. *Agronomy Journal* 51:47-49.
- Murty, B.R. 1975. Mutation breeding for resistance to downy mildew and ergot in *Pennisetum* and *Ascochyta* in chickpea. Pages 165-181 in *Induced mutations for disease resistance in crop plants (1975)*. IAEA publication no. 181. Vienna, Austria: International Atomic Energy Agency.
- Pi, C.P., and Wu, K.D. 1961. The inheritance of cytoplasmic genetic male sterility in sorghum. *Sorghum Newsletter* 4:9-10.
- Pokhriyal, S.C., Unnikrishnan, K.V., Singh, B. Ramdas, A., and Patil, R.R. 1976. Combining ability of downy mildew resistant lines in pearl millet. *Indian Journal of Genetics and Plant Breeding* 36:403-409.
- Rao, S.V. 1969. An unusual occurrence of breakdown of male sterility in bajra (*Pennisetum americanum* (Burm.) Stapf and Hubb.). *Andhra Agricultural Journal* 16:1-5.
- Reddy, B.B., and Reddi, M.V. 1970. Studies on the breakdown of male sterility and other related aspects in certain cytoplasmic male sterile lines of pearl millet (*Pennisetum typhoides* Stapf and Hubb.). *Andhra Agricultural Journal* 17:173-180.
- Ross, W.M., and Hackerott, H.L. 1972. Registration of seven iso-cytoplasmic sorghum germplasm lines. *Crop Science* 12:720-721.
- Saxena, M.B.L., and Chaudhary, B.S. 1977. Studies on the breakdown of male sterility in some male sterile lines of pearl millet (*Pennisetum typhoides*) under conditions of arid zone. *Annals of Arid Zone* 16:427-432.
- Siebert, J.D. 1982. Genetic and breeding aspects of fertility restoration in cytoplasmic male sterile pearl millet. Ph.D. thesis, University of Georgia, Athens, Georgia, USA. 151 pp.
- Simmonds, N.W. 1979. Principles of crop improvement. London and New York: Longman. 408 pp.
- Singh, A., and Laughnan, J.R. 1972. Instability of 5 male sterile cytoplasm in maize. *Genetics* 71:607-620.
- Stephens, J.C., and Holland, R.F. 1954. Cytoplasmic male-sterility for hybrid sorghum seed production. *Agronomy Journal* 46:20-23.
- Singh, S.D., Ball, S., and Thakur, D.F. 1987. Problems and strategies in the control of downy mildew. Pages 161-172, these proceedings.
- Thakur, R.P., Rao, K.V.S., and Williams, R.J. 1983a. Effects of pollination on smut development in pearl millet. *Plant Pathology* 32:141-144.
- Thakur, R.P., Talukdar, B.S., and Rao, V.P. 1983b. Genetics of ergot resistance in pearl millet. Page 737 in *Abstracts of contributed papers of the XV International Congress of Genetics, 12-21 Dec 1983, New Delhi, India Pt. 2*. New Delhi, India: Oxford and IBH Publishing Co.
- Thakur, R.P., Williams, R.J., and Rao, V.P. 1983c. Control of ergot in pearl millet through pollen management. *Annals of Applied Biology* 103:31-36.